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ASSISTANCE REQUESTED: Toxicity information for Iron (RfD)

ENCLOSED INFORMATION: Attachment 1: **PROVISONAL PEER REVIEWED TOXICITY INFORMATION FOR IRON (CASRN 7439-89-6) AND COMPOUNDS Derivation of Subchronic and Chronic Oral RfDs**

BE ADVISED: Unless specifically indicated to have been peer reviewed, it is to be noted that the attached Provisional Toxicity Value Paper(s) have not been through the U.S. EPA's formal review process; therefore, they do not represent a U.S. EPA verified assessment.

If you have any questions regarding this transmission, please contact the STSC at (513) 569-7300.

Attachments (1)

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Provisional Peer Reviewed Toxicity Values for

Iron and Compounds

(CASRN 7439-89-6)

Derivation of Subchronic and Chronic Oral RfDs

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISONAL PEER REVIEWED TOXICITY INFORMATION FOR IRON (CASRN 7439-89-6) AND COMPOUNDS
Derivation of Subchronic and Chronic Oral RfDs

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

    - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
    - California Environmental Protection Agency (CalEPA) values, and
    - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI

INTRODUCTION

A reference dose (RfD) for iron is not available on the Integrated Risk Information System (IRIS) (U.S. EPA, 2006) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2005). The Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997) reported that data regarding iron were inadequate for quantitative risk assessment. The Chemical Assessment and Related Activities (CARA) list (1991, 1994) includes a Health Effects Assessment (HEA) for Iron and Compounds (U.S. EPA, 1984) that found no reliable quantitative oral toxicity data. Iron has not been the subject of a toxicological review by the Agency for Toxic Substances Disease Registry (ATSDR) (2005) or the World Health Organization (WHO) (2005). Monographs by the International Agency for Research on Cancer (IARC) (1972, 1987), toxicity reviews by Jacobs (1977), Bothwell et al. (1979), Lauffer (1991) and Grimsley (2001), a review on dietary iron by the National Academy of Sciences (NAS) (2001), and the National Toxicology Program (NTP) (2001, 2005) management status report and chemical repository summary were consulted for relevant information. The NAS (2001) derived a Tolerable Upper Intake (TUI) level of 45 mg iron/day. The TUI is based on a minimal LOAEL of 70 mg/day (60 mg iron as ferrous fumerate plus 11 mg/day of dietary iron) identified by Frykman et al. (1994) for gastrointestinal effects and an uncertainty factor of 1.5 for use of a minimal LOAEL; a higher uncertainty factor was not used since the nature of the observed gastrointestinal effects was considered to be self-limiting. The U.S. Food and Drug Administration (FDA) promulgated a Rule in 1997 for labeling of iron-containing dietary supplements for the prevention of accidental poisoning in children (U.S. FDA, 1997). The Rule, as modified in 2003, does not contain specific exposure limits (U.S. FDA, 2003). In general, the FDA follows the NAS guidance on exposure limits for toxicity of essential elements, such as iron. Previous literature searches were conducted through September, 2001 as follows: TOXLINE (oral and inhalation toxicity and cancer from 1983 - September, 2001); CANCERLIT (1990 - September, 2001); MEDLINE (1991 - September, 2001); TSCATS, RTECS, DART/ETICBACK, EMIC/EMICBACK, HSDB, GENETOX, and CCRIS. Update literature searches were performed in October, 2005 in MEDLINE, TOXLINE (NTIS subfile), TOXCENTER, TSCATS, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents.

REVIEW OF PERTINENT LITERATURE

Iron is an essential element and deriving a risk assessment value for such chemicals poses a special problem in that the dose-adversity curve is "U-shaped". Thus, the risk value must be protective against deficiency as well as toxicity. The NAS (2001) has established guidelines for iron intake that take into account physiological differences during different life stages. For non-breast-fed infants aged 0-6 months, the NAS (2001) established a daily adequate intake (AI) for iron of 0.27 mg/day (0.04 mg/kg-day for infants 2-6 months old) based on the daily amount of iron secreted in human milk; breast-fed infants typically receive only 0.15 to 0.3 mg Fe/day. The NAS (2001) Dietary Reference Intakes (DRIs) for children are as follows: 11 mg/day (1.2 mg/kg-day) for infants between the ages of 7 and 12 months, 7 mg/day (0.54 mg/kg-day) for children aged 1-3 years, 10 mg/day (0.45 mg/kg-day) for ages 4-8 years, 8 mg/day (0.2 mg/kg-day) for ages 9-13 years and 11 mg/day (0.17 mg/kg-day) for boys and 15 mg/day (0.26 mg/kg-day) for girls aged 14-18 years. The DRI for men aged 19 years and above is 8 mg/day (0.11 mg/kg-day). The DRI for non-pregnant women is 18 mg/day (0.29 mg/kg-day) for ages between 19 and 50 years and 8 mg/day (0.13 mg/kg-day) for ages 51 years and older. The DRI for pregnant women is 27 mg/day (0.37 mg/kg-day for those aged 14-18 years and 0.35 mg/kg-day for those aged 19-50 years). The DRI during lactation is 10 mg/day (0.18 mg/kg-day) for women aged 14-18 years and 9 mg/day (0.15 mg/kg-day) for women aged 19-50 years.

According to the Centers for Disease Control and Prevention (CDC, 1998; CDC, 2005), iron deficiency is one of the most common known forms of nutritional deficiency. Its prevalence is highest among young children and women of childbearing age, particularly pregnant women. In children, iron deficiency causes developmental delays and behavioral disturbances, and in pregnant women, it increases the risk for a preterm delivery and delivering a low-birthweight baby. Young children are at great risk of iron deficiency because of rapid growth and increased iron requirements. Iron deficiency can occur due to lack of iron in the diet. If this continues, anemia results. Anemia is a manifestation of iron deficiency when it is relatively severe. Iron deficiency anemia significantly impairs mental and psychomotor development in infants and children. Although iron deficiency can be reversed with treatment, the reversibility of the mental and psychomotor impairment is not yet clearly understood. Thus, prevention and treatment need to be emphasized more than detection. In addition, iron deficiency increases a child’s susceptibility to lead toxicity. Lead replaces iron in the absorptive pathway when iron is unavailable.

In humans and other animals, levels in the body are regulated primarily through changes in the amount of iron absorbed by the gastrointestinal mucosa. The absorption of dietary iron is influenced by body stores, by the amount and chemical nature of iron in ingested food and by a variety of dietary factors that increase or decrease the availability of iron for absorption (Hillman, 2001; Santi and Masters, 2001). Iron contained in meat protein (hemoglobin and myoglobin) is absorbed intact without first being broken down to elemental iron. Non-heme iron must first be reduced to ferrous iron (Fe^{2+}) before it can be absorbed. Ferrous iron is transported across intestinal mucosal cells by active transport with the rate of transport inversely related to body iron stores. Depending upon the iron status of the body, iron is stored bound to ferritin within mucosal cells and macrophages in the liver, spleen and bone, or is transported in the plasma bound to transferrin. Serum levels of ferritin and transferrin, along with several red blood cell parameters, can be used clinically to evaluate iron balance. Although iron absorption is regulated, excessive accumulation of iron in the body resulting from chronic ingestion of high levels of iron cannot be prevented by intestinal regulation and humans do not have a mechanism to increase excretion of absorbed iron in response to elevated body levels (NAS, 1989, 2001).

Human Studies

Acute Exposure

Information on acute oral toxic doses of iron in humans is available from numerous case reports of ingestion by children, but values vary because it is difficult to obtain accurate estimates of the amount taken in most overdose situations. Reviews of these case reports indicate that doses in the range of 200-300 mg iron/kg are generally considered lethal (Arena, 1970; Krenzelok and Hoff, 1979; NRC, 1979; Engle et al., 1987; Mann et al., 1989; Klein-Schwartz et al., 1990).

Therapeutic Studies

Ferrous salts are administered orally for the therapeutic treatment of iron deficiency. The oral absorption of ferrous iron supplements is considered to be essentially the same for all ferrous salts (e.g., sulfate, fumarate, succinate and gluconate) and is approximately three times greater than that of ferric (Fe^{3+}) salts (Hillman, 2001); thus, ferric iron is not used therapeutically. Constipation and other gastrointestinal effects, including nausea, vomiting, diarrhea and gastrointestinal pain are commonly associated with administration of oral ferrous salt supplements (Hillman, 2001; Santi and Masters, 2001). Severity of effects is variable, ranging from mild to severe, and depends upon dose and individual susceptibility. The onset of symptoms typically occurs at the initiation of treatment and continues throughout the duration of treatment. Although there is no indication that the severity of gastrointestinal effects varies over the course of treatment, severity is decreased in some patients when iron supplements are administered with food (Hillman, 2001; Santi and Masters, 2001). For most patients, iron deficiency is reversed within six months of treatment, thus limiting the duration of exposure.

The mechanism of iron-induced gastrointestinal toxicity is not established, although it is postulated that adverse effects are due to irritant effects of the free iron ion on the gastric muscosa (Liguori, 1993). The role of absorbed iron in the development of gastrointestinal adverse effects is unknown. The adverse effects of exposure to oral iron supplements has been investigated in several studies (Blot et al., 1981; Brock et al., 1985; Coplin et al., 1991; Fryklman et al., 1994; Hallberg et al., 1966; Liguori, 1993).

Frykman et al. (1994) evaluated the adverse effects of daily oral therapy with iron fumarate in a double-blind, crossover, placebo-controlled study in Swedish male [n=25; mean age 45 years (range 40-52)] and female [n=23; mean age 41 years (range 34-45)] adult blood donors. Study subjects were administered 60 mg elemental iron as a daily dose of iron fumarate for one month, with each study subject serving as their own placebo control. Compared to the placebo treatment period, the percentage of subjects reporting constipation (placebo 20%, ferrous fumarate 35%, $p<0.05$) and total gastrointestinal symptoms (nausea, obstipation, gastric pain and diarrhea (placebo 14%, ferrous fumarate 25%, $p<0.01$) was significantly increased during ferrous fumarate treatment. Although the severity of gastrointestinal effects was graded as minor in most study subjects, four subjects withdrew from the study due to severe gastrointestinal symptoms associated with iron fumarate. In a matched group of 49 adults taking a daily combination supplement of porcine-derived heme-iron and iron fumarate containing a total daily supplement of 18 mg iron/per day, the frequency of gastrointestinal symptoms was not increased compared to placebo. No differences in therapeutic efficacy, as measured by serum ferritin and hemoglobin levels, were observed between the non-heme iron and heme-iron treatment groups.

Adverse effects of four oral iron preparations were evaluated in 1496 male and female adult blood donors in a series of double-blind, placebo controlled trials (Hallberg et al., 1966). The following treatment groups were compared: (1) placebo (195 subjects) and ferrous sulfate (198 subjects; 222 mg elemental iron/day); (2) placebo (199 subjects), ferrous sulfate (120 subjects; 222 mg elemental iron/day), ferrous fumarate (118 subjects, 222 mg elemental iron/day), and ferrous gluconate (120 subjects; 222 mg elemental iron/day); and (3) placebo (200 subjects), ferrous sulfate (195 subjects; 180 mg elemental iron/day), ferrous glycine sulfate (200 subjects; 180 mg elemental iron/day), and ferrous gluconate (196 subjects; 180 mg elemental iron/day). Treatments were administered for two weeks. For all iron treatments, the frequency of adverse gastrointestinal effects was significantly increased compared to the matched placebo group ($p<0.05$). Adverse effects reported include constipation, diarrhea, heartburn, nausea and epigastric pain. No statistically significant differences in the frequency of adverse effects were observed between iron treatments for subjects receiving 222 mg elemental iron/day or between iron treatments for subjects receiving 180 mg elemental iron/day. In the seven iron treatment groups, the percentage of subjects reporting gastrointestinal effects ranged from 22.9% in the 222 mg ferrous sulfate group to 31.5% in the 222 mg ferrous gluconate group. In the three placebo treatment groups, the percentage of subjects reporting gastrointestinal effects ranged from 12.4 to 13.6%. Although statistical comparisons were not made between the 180 and 222 mg iron/day treatments, the frequency of adverse effects was similar for all iron treatment groups.

Gastrointestinal symptoms were reported in pregnant women treated daily with oral iron supplements containing 105 mg elemental iron and 500 mg ascorbic acid (55 women) or 105 mg elemental iron, 500 mg ascorbic acid and 350 mg folic acid (54 women) during the third trimester of pregnancy (Blot et al., 1981). The form of iron was not reported. No placebo control group was included. Gastrointestinal adverse effects reported include nausea, diarrhea, constipation and epigastric pain. Approximately 16% of all patients reported minor gastrointestinal symptoms, 14% reported severe effects and 6% stopped treatment due to adverse effects. Adverse effects occurred with approximately the same frequency in the two treatment group, although data were not reported.

The tolerability of iron protein succinylate and ferrous sulfate were compared in a double-blind clinical trial in 1095 patients with iron deficiency (Liguori, 1993). Patients received daily treatment with a controlled-release formulation of ferrous sulfate containing 105 mg elemental iron (64 males and 485 females) or iron protein succinylate containing 120 mg elemental iron (55 males and 491 females) for 60 days. No placebo control group was included. In the ferrous sulfate group, 26.3% of patients reported adverse gastrointestinal effects (heartburn, epigastric pain, constipation and abdominal pain), compared to 11.5% of patients treated with iron protein succinylate ($p<0.05$).

The adverse effects of oral treatment with a conventional ferrous sulfate tablet were compared to a ferrous sulfate wax-matrix tablet in a single-blind, parallel group study in 543 subjects (Brock et al., 1985). No placebo control group was included. Subjects were administered a conventional ferrous sulfate table containing 50 mg elemental iron/day (272 subjects) or a sulfate wax-matrix tablet containing 50 mg elemental iron/day (271 subjects) for 56 days. Approximately 45% of subjects treated with conventional ferrous sulfate reported moderate-to-severe gastrointestinal effects, including abdominal discomfort, nausea, vomiting, constipation and diarrhea, compared to approximately 17% of subjects treated with the ferrous sulfate wax-matrix preparation, a statistically significant difference ($p<0.001$).

The tolerability of ferrous sulfate (50 mg elemental iron/day) and bis-glycino iron II (50 mg elemental iron/day) was compared in a double-blind, crossover trial in 42 women (Coplin et al., 1991). The treatment period for each iron supplement was two weeks. No placebo treatment period was included. The frequency of adverse gastrointestinal effects (abdominal pain, bloating, constipation, diarrhea and nausea) was similar for the two treatments, with 54% and 59% of subjects reporting gastrointestinal symptoms during treatment with bis-glycino iron II and ferrous sulfate, respectively. The difference between treatments was not statistically significant.

Effects of iron therapy on the upper gastrointestinal tract were evaluated in 14 healthy volunteers [13 women, 1 man; mean age 29 years (range: 24-48 years)] who were instructed to ingest 325 mg tablets of ferrous sulfate (119.5 mg elemental iron) three times/day before meals (358.5 mg elemental iron/day) for 2 weeks (Laine et al., 1988). Evaluation consisted of a gastrointestinal symptom survey, qualitative (Hemoccult) and quantitative (HemoQuant; mg mercury/g stool) testing for fecal blood loss, endoscopy of the upper gastrointestinal tract and histological examination of pinch biopsies of the gastric body, antrum and duodenum. Based on actual average ingestion of 2.5 tablets/day (2-week study) and 2.6 tablets/day (1-week study) and a reference human body weight of 70 kg (U.S. EPA, 1987), the estimated doses consumed by the subjects were 4.3 and 4.4 mg iron/kg-day, respectively, in addition to dietary iron. Compared to baseline measurements in the two weeks prior to treatment, all subjects had significantly increased ($p<0.05$) dark brown-black stools and symptoms of nausea and vomiting during the treatment period, but not abdominal pain. Hemoglobin levels in stool did not change significantly after iron treatment. Endoscopic examination showed a significant ($p=0.003$) increase in abnormalities in the stomach, but not duodenum, after therapy. These changes consisted of erythema, small areas of subepithelial hemorrhage and solitary antral erosions in nine, six and two subjects, respectively, and were considered only minimally abnormal. No treatment-related histological changes were observed. Although it was speculated that the changes in the stomach could represent a mild form of iron poisoning, the investigators concluded that the treatment caused mild endoscopic abnormalities of uncertain clinical significance in the stomach. Evidence for iron overload (tissue biopsies or hematologic iron status indices) was not examined. Considering additional dietary exposure, an exposure level of about 4.3 mg/kg-day represents, at worst, a minimal LOAEL.

Adverse developmental effects in humans have not been associated with the ingestion of supplemental iron during pregnancy. As indicated above, NAS (2001) recommended that pregnant women supplement their diets with 27 mg iron/day (0.35 mg/kg-day). McElhatton et al. (1991) reported on 49 women who took an overdose of a simple iron preparation (53%) or iron with folate preparation (47%). In 48 of the women, the amount of iron ingested was known; 28 took > 1.2 g and the remainder took 1.2 g. There were 25 women who received chelation treatment with desferrioxamine (DFO) and 12 who received an emetic. Maternal toxicity, consisting of nausea, vomiting, hematoemesis, abdominal pain and diarrhea, was observed in 35 of the women. Two spontaneous abortions occurred and there were three premature deliveries. One of the spontaneous abortions and the premature deliveries were not related to the iron overdose. It is not known if the other spontaneous abortion occurring at 22 weeks (3 weeks after the overdose) was caused by the iron overdose. No conclusions on the developmental toxicity of iron can be made.

Chronic Exposure

While chronic iron toxicity occurs in people with genetic metabolic disorders resulting in excessive iron absorption or abnormal hemoglobin synthesis, or who receive frequent blood transfusions (Jacobs, 1977; Bothwell et al., 1979), there is a long-standing controversy as to whether a chronic overload due to oral intake is possible in individuals with a normal ability to control iron absorption (Hillman and Finch, 1985). Nevertheless, "the cumulative experience in human subjects suffering from iron overload of various etiologies strongly suggests that iron is noxious to tissues [when]...present in parenchymal cells...for a sufficiently long period of time" (Bothwell et al., 1979).

Looker et al. (1988) made comparisons of dietary iron intake and biochemical indices of iron status based on values taken from the second National Health and Nutrition Examination Survey (NHANES II) data base[1]. NHANES II was a probability sample of the noninstitutionalized U.S. population aged 6 months to 74 years, conducted between 1976 and

[1] The latest version of this data base, NHANES III (1984-1988) evaluated 30,000 subjects aged 2 months and above (NAS, 2001). Despite minor differences in the data sets, the conclusions drawn by Looker et al. (1988) based on NHANES II appear to be valid for the NHANES III data.

1980 by the National Center for Health Statistics. These data suggest that normal intake of iron by men 16-74 years old exceeds the DRI, and that iron intake is somewhat lower than the DRI for women younger than 51 years. Concomitant with the study of dietary intake, the NHANES II measured the iron status of these populations. The percent serum transferrin saturation, a measure of the residual capacity of the iron transport system to process potential variations in iron from dietary intake or catabolized body stores, ranged from 24% saturation for pre- and post-menopausal women not using iron supplements to 29% saturation for adult male supplement users. These values are within the normal range (20-40%). The Looker et al. (1988) evaluation of the NHANES II iron status data concerned iron deficiencies, only, and did not address iron overload directly. However, iron overload conditions would likely be evidenced by increased saturation of serum transferrin and increased serum ferritin concentrations, which were also within the normal range. Therefore, the corresponding dietary intakes are presumed to represent chronic NOAELs. Looker et al. (1988) estimated daily iron intakes ranging from 10.0 for elderly women to 18.7 mg/day for young adult men in the study population. These daily intakes correspond to a range of about 0.15 to 0.27 mg/kg-day, depending on assumptions of average body weight. Taking the highest intake level of 18.7 mg/day and a body weight of 70 kg, a NOAEL of 0.27 is established for chronic iron toxicity.

Hemosiderosis (or siderosis) and iron overload are increases in tissue iron or a general increase in iron stores without associated tissue damage (Bothwell et al., 1979; Jacobs, 1977). Hemochromatosis describes massive iron overload (15 g of body iron stores or greater) together with cirrhosis and/or other tissue damage attributable to iron. Although focal deposits of iron may occur in any part of the body where red cells are extravasated, the clinical syndrome of hemochromatosis typically involves damage to the hepatic parenchyma (particularly fibrosis), heart (cardiac dysfunction including failure) and endocrine glands (particularly hypogonadism). Pancreatic iron deposition is common and massive deposits may be associated with fibrosis and diabetes. A number of studies involving chronic oral administration of iron to animals have been designed in an attempt to identify an animal model for hemochromatosis. Most of these studies have been negative (Bothwell et al., 1979; NRC, 1979). Animal studies involving parenteral administration of iron have been generally negative as well, even though parenteral routes bypass the mechanisms that regulate absorption of iron from the gastrointestinal tract.

Chronic iron toxicity has been observed in people with idiopathic hemochromatosis (a genetic metabolic disorder resulting in excessive iron absorption), abnormalities of hemoglobin synthesis (e.g., thalassemia) or various anemic states (e.g., sideroblastic anemia), frequent blood transfusions or a combination of these conditions (Jacobs, 1977; Bothwell et al., 1979). Chronic hemochromatosis has also occurred among the South African Bantu population from an excessive intake of absorbable iron in an alcoholic beverage.

Habitual excessive intake of iron by the Bantus is attributed to consumption of home-brewed Kaffir beer, which was contaminated by iron vessels during brewing (Bothwell and Bradlow, 1960; Bothwell et al., 1964). The beer's high acidity (pH 3-3.5) enhanced iron leaching from the vessels. The iron in the beer is readily assimilable (i.e., ionizable) due to the acidity and presence of iron-complexing ligands such as fructose, and is absorbed to approximately the same degree as ferric chloride. The alcohol content of the beer is also believed to contribute to the bioavailability of the iron (Jacobs, 1977; Finch and Monsen, 1972). Based primarily on drinking habits and analyses of beer samples, the estimated average dietary iron intake of the Bantu men ranged from 50-100 mg/day from beer alone (Bothwell et al., 1964). Using a reference body weight of 70 kg (U.S. EPA, 1987), this range corresponds to 0.7-1.4 mg/kg-day. Histological examinations of the liver of 147 Bantus (129 male, 18 female) ranging in age from 11-70 years (most were between 20 and 50 years old) that died from acute traumatic causes were performed (Bothwell and Bradlow, 1960). Varying degrees of hepatic siderosis were observed in 89% of the cases; the degree tended to increase with age 40-50 years or less. The siderosis was mild in 59% and severe in 19% of the cases, respectively. There was a close correlation between hepatic iron concentration and portal fibrosis and cirrhosis. Although the overall prevalence was low (15.6% fibrosis and 1.4% cirrhosis), all 11 subjects with the highest iron concentrations (>2.0% dry weight of liver) showed either fibrosis or cirrhosis. Histological examination of the spleen (50 subjects) also showed siderosis and unspecified histological changes. Malnutrition and alcoholism could have played a role in the etiology of the hepatic and splenic siderosis in the Bantus. A NOAEL in the range of 0.7 - 1.4 mg/kg-day is indicated but may be low given the likely higher bioavailability of iron in the beer than for normal dietary exposure. Given the generally poor nutritional health status of this population, the relevance of this study for application to the U.S. population is questionable.

Ethiopia reportedly has the highest per capita iron intake in the world, with an average daily intake of 471 mg iron/day (range 98-1418 mg/day; 1.4-20.3 mg iron/kg-day assuming 70 kg body weight) (Roe, 1966; Hofvander, 1968). Increased stored iron in the liver and adverse health effects have not been observed due to low bioavailability of the iron in Ethiopian food.

A few studies have suggested that high iron intake may be a risk factor for myocardial infarction (Salonen et al., 1992; Lauffer, 1991; Sullivan, 1992). Five other large studies found no association between serum ferritin levels and coronary heart disease (NAS, 2001). Various other measures of iron status (serum transferrin saturation, serum iron concentration and total iron-binding capacity) have been examined for a possible link to cardiovascular disease in prospective cohort studies, but results overall have been characterized as contradictory (Meyers, 1996; NAS, 2001). The NAS (2001) concluded that the available evidence "does not provide convincing support for a causal relationship" between the level of dietary iron intake and the risk for coronary heart disease, although iron cannot be definitively excluded as a risk factor.

Animal Studies

Repeated-dose oral studies in experimental animals found no significant effect of treatment with inorganic iron compounds. No treatment-related adverse changes in clinical signs, body or organ weights, food consumption or histopathology were observed in male Sprague-Dawley rats that had daily dietary intakes of 35, 70 or 140 mg of iron (as $FeSO_4$ or FeEDTA) per kg for up to 61 days (Appel et al., 2001). In male and female F344 rats that were exposed to drinking water containing 0.25 or 0.5% ferric chloride ($FeCl_3 \cdot 6H_2O$) for 104 weeks, there were no dose-related effects other than reduced water intake (possibly affected by palatability) and body weight gain (Sato et al., 1992). In the latter study, the iron intakes were 58 or 110 mg/kg-day in males and 65 or 116 mg/kg-day in females.

No treatment-related teratogenic or embryotoxic effects were observed in rats given 2.7 mg iron/kg-day as ferric chloride on gestational days 6-15 (Nolen et al., 1972), or in rats and mice given 24-76 mg iron/kg-day as ferrous sulfate for 6 days during gestation (days unspecified) (Tadokoro et al., 1979). Some embryonic mortality (numbers and species not reported) occurred in the latter study at 240 mg iron/kg-day.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR IRON

Iron is an essential element, as such, the RfD must be protective against both toxicity and deficiency. Using the values for dietary intake and iron status indices taken from the second National Health and Nutrition Examination Survey (NHANES II) data base, it is possible to establish a NOAEL for chronic toxicity. Looker et al. (1988) made comparisons of dietary iron intake and biochemical indices of iron status using data from NHANES II. The average intakes of iron ranged from 0.15 to 0.27 mg/kg-day. The serum ferritin levels and percent serum transferrin saturation were within the normal range. Thus, intake levels of 0.15-0.27 mg/kg-day are sufficient to protect against iron deficiency. However, the NHANES II data do not provide information to identify daily dietary iron intakes associated with toxicity. Therefore, daily dietary iron intakes were not considered as the basis for the p-RfD.

Most of the quantitative chronic oral toxicity data for iron have been obtained from studies of the Bantu population of South Africa. These data indicate that intakes in the range of 0.7-1.4 mg iron/kg-day in home-brewed beer are associated with hemosiderosis and liver cirrhosis (Bothwell and Bradlow, 1960; Bothwell et al., 1964). However, confounding factors such as malnutrition and unusually high iron bioavailability due to the high acidity and ethanol in the beer preclude use of these data for risk assessment. Much higher dietary intakes (average 6.7 mg/kg-day) of less soluble forms of iron are tolerated in non-western diets as indicated by studies of populations in Ethiopia. Thus, although toxicity associated with iron overload due to chronic oral intake can be demonstrated qualitatively or even semiquantitatively, assignment of a precise LOAEL for normal individuals consuming western diets is compromised by studies containing confounding factors.

Gastrointestinal toxicity, which is commonly associated with the therapeutic use of iron supplements, was identified as the critical effect for the basis of the provisional subchronic and chronic RfDs. The most frequently reported symptoms include epigastric pain, nausea, vomiting, constipation and diarrhea. Several prospective clinical trials in healthy subjects and iron-deficient patients identify a LOAEL for gastrointestinal toxicity of 50 to 180 mg elemental iron/day; NOAELs were not established (Blot et al., 1981; Brock et al., 1985; Coplin et al., 1991; Frykman et al., 1994; Hallberg et al., 1966; Liguori, 1993). The treatment durations in these studies range from 2 weeks to approximately 3 months. Although no chronic exposure studies reporting gastrointestinal toxicity were identified, clinical experience with iron supplements indicates that gastrointestinal effects are associated with oral iron therapy, regardless of the duration of treatment and that symptom intensity does not change over the course of treatment (Hillman, 2001; Santi and Masters, 2001). This observation suggests that the response is related to the concentration of iron in the intestinal tract and not to the time-integrated dose. Therefore, gastrointestinal toxicity is considered as the critical effect for both the subchronic and chronic p-RfDs.

The lowest LOAEL of 50 mg elemental iron/day for gastrointestinal toxicity associated with iron supplements was reported in two studies that did not use a placebo-controlled design (Brock et al., 1985; Coplin et al., 1991); therefore, data were not considered suitable for derivation of the p-RfD. The placebo-controlled, cross-over design study by Frykman et al. (1994) reporting a LOAEL of 60 mg/day in Swedish men and women was identified as the critical study. Results of this study show that daily treatment with ferrous fumarate (60 mg elemental iron/day) for one month produced a statistically significant increase in gastrointestinal effects compared to placebo. To determine the LOAEL for total daily iron intake, the LOAEL for daily supplementation with ferrous fumarate of 60 mg elemental iron/day was added to the estimated mean dietary intake for six European countries of 11 mg elemental iron/day (NAS, 2001) for a total daily iron intake of 71 mg elemental iron/day. Based on a reference body weight of 70 kg (U.S. EPA, 1987), the LOAEL for gastrointestinal effects for total daily iron intake is 1 mg elemental iron/kg-day. This LOAEL is considered to be a minimal LOAEL because gastrointestinal effects were characterized by most study participants as minor in severity.

The provisional subchronic and chronic RfD for iron was derived from the LOAEL of 1 mg/kg-day for total daily iron intake for adverse gastrointestinal effects as follows:

$$\begin{aligned}\textbf{p-RfD (subchronic and chronic)} &= \textbf{LOAEL} \div \textbf{UF}\\ &= \textbf{1 mg/kg-day} \div \textbf{1.5}\\ &= \textbf{0.7 mg/kg-day}\end{aligned}$$

Dividing the LOAEL of 1 mg/kg-day by an uncertainty factor of 1.5 yields a subchronic and chronic p-RfD of 0.7 mg/mg-day. The uncertainty factor of 1.5 includes the individual uncertainty factors of 1.5 for use of a minimal LOAEL, 1 for sensitive individuals, 1 for less than lifetime exposure, and 1 for an adequate data base. An uncertainty factor of 1.5 was applied to account for extrapolation from a minimal LOAEL to a NOAEL for a non-serious effect. A higher uncertainty factor for use of a minimal LOAEL was not used since the observed gastrointestinal effects are not considered serious and are reversible when exposure is discontinued. Furthermore, gastrointestinal symptoms are not associated with dietary intake of similar levels of iron (NAS, 2001). Because individuals sensitive to gastrointestinal symptoms are considered to be included in the studies investigating effects of therapeutic iron; an uncertainty factor of 1 for sensitive individuals results. An uncertainty factor of 1 was used to account for less than lifetime exposure. Although exposure duration in the Frykman et al. (1994) study was only one month, there is no evidence to suggest that symptoms increase with longer exposure periods. An uncertainty factor of 1 was used to reflect an adequate database in humans, due to the extensive use of therapeutic iron.

Except for individuals with disorders of iron metabolism, little information is available on the long-term systemic toxicity of orally ingested iron. This assessment, therefore, focuses more on what is known to be a safe oral intake of iron for the general human population (i.e., apparently healthy normal individuals). The provisional reference dose is estimated to be an intake for the general population that is adequately protective from adverse health effects. Further, it is also important to note that individual requirements for, as well as adverse reactions to, iron may be highly variable. Some individuals may, in fact, consume a diet that contributes more than the provisional reference dose, without any cause for concern. In addition, specific population subgroups may have higher nutritional requirements than the provisional RfD would provide. The p-RfD may not be protective of individuals with inherited disorders of iron metabolism or other conditions which affect iron homeostasis.

This assessment is essentially the same as that proposed by Stifelman et al. (1995).

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